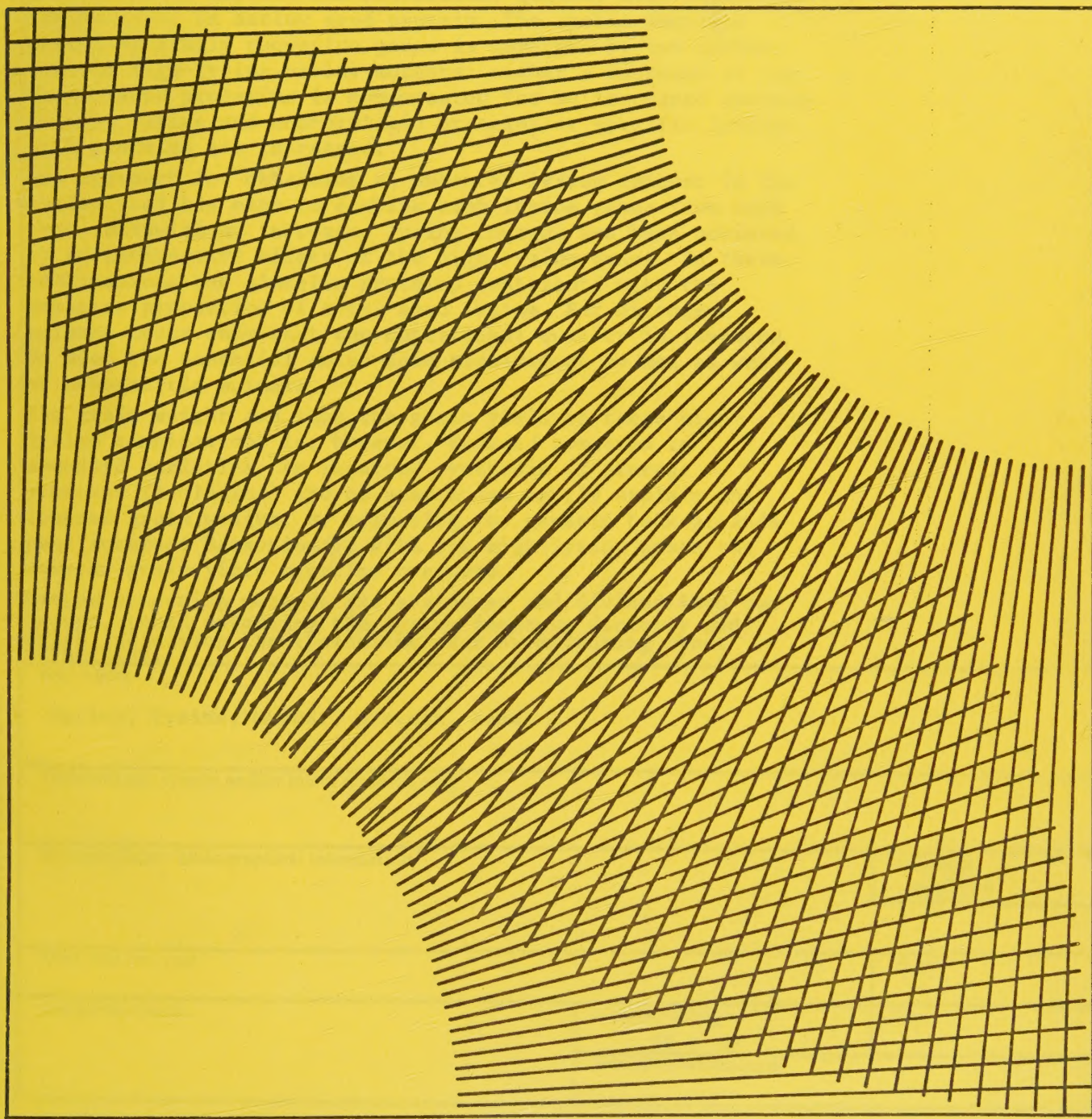


Characterization of high-lysine barley genotypes

Anneli
Tallberg



1981

CHARACTERIZATION OF HIGH-LYSINE BARLEY GENOTYPES

av

Anneli Tallberg

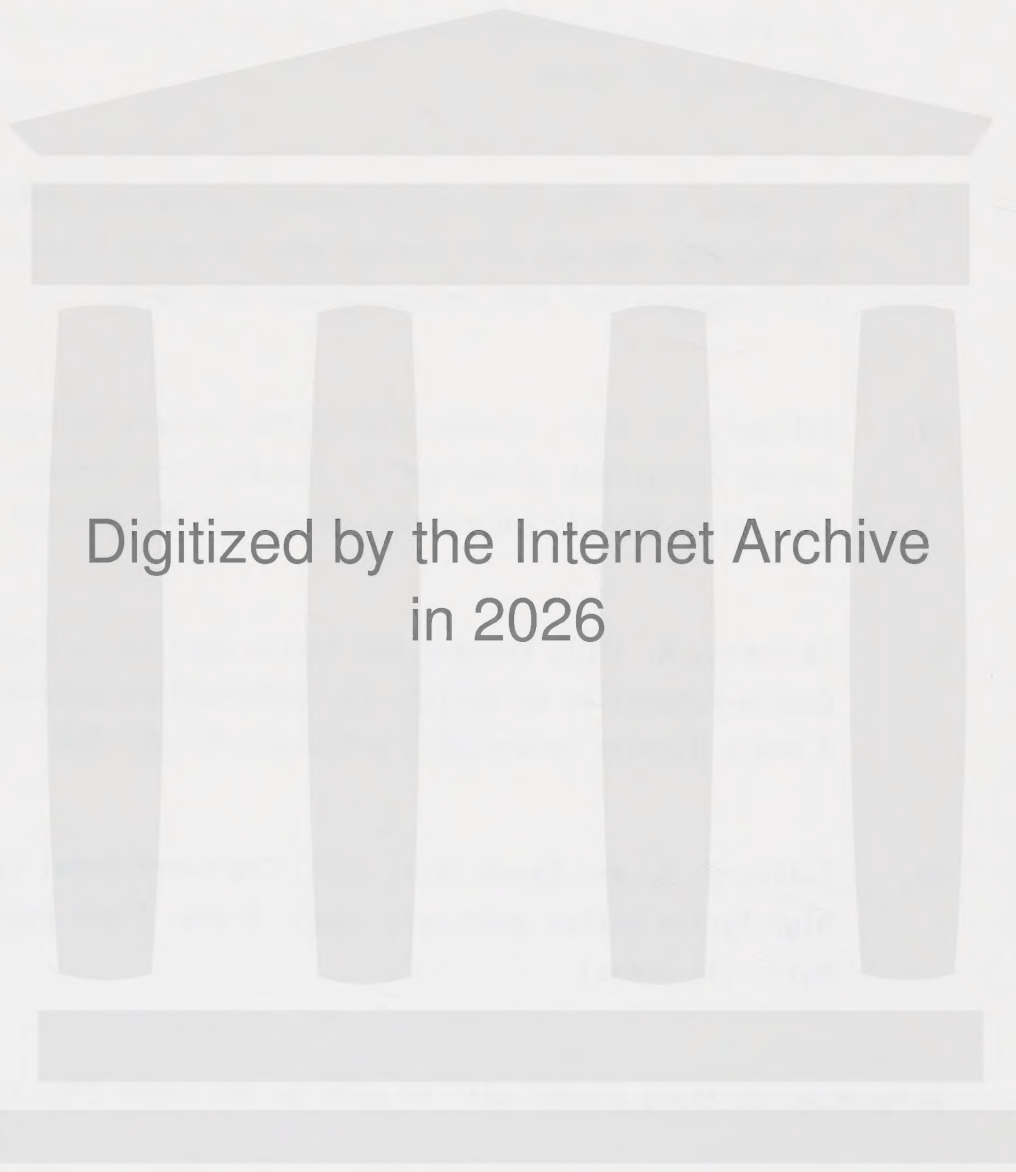
Fil.kand., Mlm.

Akademisk avhandling som för avläggande av filosofie doktorsexamen vid matematisk-naturvetenskapliga fakulteten vid Universitetet i Lund kommer att offentligen försvaras å Genetiska institutionens föreläsningssal fredagen den 9 oktober 1981 kl. 10.00 fm.

The thesis is based on the following papers:

- I. Tallberg, A. 1973. Ultrastructure and protein composition in high-lysine barley mutants. *Hereditas* 75: 195-200
- II. Tallberg, A. 1977. The amino acid composition in endosperm and embryo of a barley variety and its high-lysine mutant. *Hereditas* 87: 43-46
- III. Tallberg, A. 1980. Comparison between screening methods for lysine with the use of a barley material with a varying amino acid composition. *Acta Agric. Scand.* 30: 26-32
- IV. Tallberg, A. 1981. Protein and lysine content in high-lysine double-recessives of barley. I. Combinations between mutant 1508 and a Hiproly back-cross. *Hereditas* 94: 253-260
- V. Tallberg, A. 1981. Protein and lysine content in high-lysine double-recessives of barley. II. Combinations between mutant 7 and a Hiproly back-cross. *Hereditas* 94: 261-268
- VI. Tallberg, A. and Eggum, B.O. 1981. The nutritional value of high-lysine barley genotypes. *Qual. Plant. Plant Foods Hum. Nutr.* (in press)

References to these papers will be made by the Roman figures above.



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Table of contents:	Page
INTRODUCTION	1
THE CEREAL SEED PROTEINS	2
PATHWAYS TO IMPROVE THE LYSINE CONTENT IN CEREAL SEEDS	5
LYSINE SCREENING METHODS	20
BIOLOGICAL EXPERIMENTS - FEEDING TRIALS	25
YIELDING PROBLEMS	26
FINAL CONCLUSIONS	29
SOME FUTURE PROSPECTS	29
ACKNOWLEDGEMENTS	30
LITERATURE CITED	31

INTRODUCTION

The seeds of cereal plants constitute a major energy source in the diet of man and domestic animals. Malnutrition arisen from growing populations and the scarcity of energy in the developing countries has increased the demand for calories. This has been met by an increased production of cereals over legumes, since cereals are first and foremost a source of calories (carbohydrates). For people in the developing countries the protein supply is therefore practically emanating from cereals only. The increased cereal production, at the expece of a legume production, has meant an increase in production of protein with poorer quality, as legumes and cereals complement each other with regard to certain essential amino acids required by man. This poor protein quality of cereal seeds is mainly due to a low content of lysine, which is often the first limiting amino acid in cereal grain proteins.

In the developed countries the increased consumption of animal protein has increased the demand for lysine to gain optimum protein efficiency. This requires more grain and more protein concentrates (e.g. soybean protein). The developed countries will therefore become major competitors for the food and protein supply that could be used directly for human consumption in the developing countries. This is now diverted to animal production, which means that for the developing as well as for the developed countries, there is a great demand for cereal protein with a better quality. How can plant breeding contribute to alter this state? One approach is to improve the protein quality in cereals by changing the relative composition of proteins in the seed, favouring an increase of proteins rich in lysine.

The discovery of opaque-2 and floury-2 maize (Mertz et al. 1964; Nelson et al. 1965) with raised lysine levels in the seed gave an impetus to searching for varieties with improved protein quality in other cereals. The high-lysine barley variety, Hiproly, was found in the World Barley Collection (Munck et al. 1970) and was followed by the recognition of a high-lysine sorghum variety (Singh and Axtell 1973). The finding of high-lysine cereal genotypes in world collections has encouraged the search for improved protein quality in cereal seeds by means of muta-

genic treatments. Some of the most successful work in this field has been performed at Risø, Denmark, where several barley mutants with a wide range of lysine content have been identified (Doll 1973, 1976).

Barley grain, in Sweden the most used cereal for animal production, has a poor protein quality. If the lysine content in the barley seed protein is increased without reduction in yield, it is possible to decrease the import of high quality protein, which in turn would be beneficial for the political economy and might lessen some of the pressure on the developing countries. A screening program at Svalöf was set up for identification of barley varieties with an increased lysine level in the seed protein. The recognition of the Ethiopian barley variety, Hiproly, with an increased lysine as well as protein content in the seed, and the tentative role it could play in breeding for improved protein quality has been discussed extensively by Munck (1972). Later on, several high-lysine barley mutants have been identified and some of them have been used for breeding aims.

In this paper there will be evaluated some of the results reported from high-lysine barley studies, with emphasis on the investigations of double-recessives, e.g., combinations of two high-lysine varieties. There will be discussed the nature of the changes that have taken place in the seed protein fractions of some high-lysine barley genotypes, the differences that exist in the ultrastructure and biochemistry of the seed and the implications for the nutritional value. Furthermore, different methods for lysine screening will be presented. Finally, future research pertaining to solve the yielding problems of high-lysine barley will be commented upon.

THE CEREAL SEED PROTEINS

To understand the changes in seed protein composition associated with the improved high-lysine barley genotypes a brief outline of the different protein classes is presented in Table 1 and, below, described in more detail.

Table 1. Characterization of cereal seed proteins

Class	Solubility	Function
Albumins and globulins	Water and salt solutions	Soluble, enzymic and metabolic proteins
Prolamins	Alcohol solutions + reducing agent	Storage proteins acting as a reserve and deposited in protein bodies
Glutelins	Alkali solutions + reducing agent + dissociating agent	Bound structural proteins associated with membranes and corresponding to the "matrix" protein of the cell

The composition and the amount of protein in the cereal seed determine the nutritional quality of the protein. Therefore it is important that the methods are adequate for separating and comparing the proteins. The separation and characterization of cereal seed proteins has almost always been based upon their solubility properties. Osborne (1924) classified the proteins as albumins, globulins, prolamins and glutelins by sequential extraction with water, dilute salt solutions, aqueous alcohols and alkaline extractants, respectively.

The albumin and globulin proteins, often extracted as a single "salt-soluble" fraction, are the main constituents of the seed embryo and aleurone layer. These are mostly the enzymes involved in growth and metabolism of the seed and are rich in lysine. Prolamins and glutelins, the remainder of the protein after salt extraction, are primarily derived from the endosperm. Kirkman et al. (1981) have shown that any nitrogen in excess of that needed for seed metabolism in barley is diverted into the prolamins, which means that the prolamins fraction acts as a storage protein, while this is not considered for the glutelin fraction.

The prolamins can thus, in accordance with Miflin (1978), be defined as follows:

- they
- perform a true storage function, i.e. they are accumulating excess nitrogen not required in cell metabolism
 - are deposited in protein bodies
 - consist of a number of polypeptides which are genetically determined
 - are soluble in alcohol solvents because of their hydrophobic properties
 - are poor in lysine but have a large number of glutamyl and proline residues.

This definition is adequate for the prolamins of barley, maize, wheat and sorghum but not for the storage proteins of rice and oats, which apparently do not have the solubility characteristics of prolamins (Miflin 1978). Because of incomplete extraction of prolamins by Osborne fractionation, misleading values for the total amount and the amino acid composition of the glutelin fraction might be obtained. For a complete extraction of barley prolamins Shewry et al. (1978) point out the necessity of using a reducing agent in the alcohol solvent and to rigorously stir the meal suspension under hot conditions.

The barley prolamins, called hordeins, have been studied in detail by different electrophoretic techniques. On the basis of these results in genetic studies the hordeins are divided into two major groups of polypeptides, hordein-1 and hordein-2, which are coded for by genes in two corresponding loci, Hor 1 and Hor 2 (Doll and Brown 1979).

The glutelin fraction, composed of a heterogenous mixture of proteins, has always been difficult to define and measure because of inadequate extraction methods, which do not ensure a complete prolamins extraction.

These endosperm proteins having strong associative tendencies are easier solubilized when the alkaline solution is supplemented with a reducing and dissociating agent. Only if the extraction of albumins, globulins and prolamins is complete, the subsequent fraction solved in alkaline solutions can be considered a glutelin. Under these conditions (Mifflin and Shewry 1979a) the glutelins can be defined as a class of proteins that do not have a prolamins-like amino acid composition:

- they
- are rich in lysine and relatively poor in glutamyl residues and proline
 - are unlikely to be storage proteins
 - are probably membrane proteins.

Cereal grains seem to pass through a fixed pattern of development first exemplified in barley by Bishop (1930). During the first phase of seed development the albumins and globulins needed for cell metabolism are formed. After two weeks the synthesis of prolamins and glutelins accelerates and these proteins become the major components after c. 3-4 weeks.

PATHWAYS TO IMPROVE THE LYSINE CONTENT IN CEREAL SEEDS

As displayed by the different high-lysine cereals the lysine content of the seed protein can be increased in different ways. Theoretically, the following mechanisms could be identified in raising the lysine level of the seed protein.

- A. Change of amino-acid composition of the central endosperm
 1. Through a decrease of lysine-poor proteins, prolamins, resulting in increased amounts of free lysine and non-prolamins proteins.
 2. Through an increase of certain lysine-rich proteins.
 3. Through an increased lysine content in the lysine-poor prolamins fraction.
- B. Morphological changes in the seed
 1. An increased embryo/endosperm weight ratio.

2. An increased number of aleurone layers.

C. Interactive effect of combined high-lysine factors.

The proceeding characterization of the high-lysine barley mutants and lines will follow this scheme.

A1 - decrease of lysine-poor prolamins and increase of non-prolamin proteins

The increased lysine content in the seed protein of the high-lysine barley mutants is generally caused by a reduction of the prolamin fraction compensated for by an increase of non-prolamin fractions. Among the Risø mutants of this type mutant 1508 and mutant 7 show the highest and the lowest lysine increase, respectively.

Mutant 1508 has an increased lysine content of 45% in protein compared to its mother variety Bomi (Ingversen et al. 1973; Doll et al. 1974), thus almost reaching the optimal level of 5.44 g lysine/16 g N recommended for cereal diets by FAO. The raised lysine content is conditioned by a single recessive gene, lys3a, exclusively expressed in the endosperm (II) and drastically changing the protein composition of the mutant. The prolamin fraction is reduced with c. 65% compared to Bomi, while the salt-soluble and glutelin fraction as well as free lysine is increased (Ingversen et al. 1973; Brandt 1976; Mifflin and Shewry 1979b). Despite the great variation of extraction procedures used, the relative reduction of hordein is the same in all the studies, but the total values in percent of total protein ($N \times 6.25$) range between 29-59 for Bomi and 9-19 for mutant 1508. Comparison of total values thus should be made with caution when differing extraction procedures are used. An extensive comparison of different extraction methods and the various values obtained in high-lysine barley lines, is presented by Mifflin and Shewry (1979b).

The amount of lysine in the hordein fraction of mutant 1508 is 200% above that of Bomi - 2.2% compared to 0.7% - while the lysine content of the other fractions are unaltered - c. 5.2% in both salt-soluble and glutelin proteins (Shewry et al. 1980b). Despite this enormous relative lysine increase in mutant 1508 hordein, this fraction accounts for only 5-10% of total lysine in the seed. This depends on the reduction of the hordein fraction as well as the very low total amount of lysine in this fraction

compared to the others. Comparison of the hordein composition between mutant 1508 and Bomi by different electrophoretic techniques reveals changes in both the nature and the distribution of the polypeptides in the mutant.

As shown by Kreis (1979) the starch synthesis pathway in mutant 1508 is blocked, leading to a much higher than normal sucrose accumulation. No clear-cut enzymatic deficiencies in the starch synthesis have been detected, that could explain this interference in the carbohydrate metabolism of the mutant. Kreis (1979) has put forward the hypothesis that a normal storage protein synthesis is essential for a normal starch synthesis, because of the interference of non-stored nitrogen (free amino acids) in the carbohydrate metabolism. Further investigations of these deleterious effects of the mutant 1508 seed are hence needed for an exact biochemical explanation of the relationship between hordein and starch synthesis.

The hordein as well as the starch deficiency in mutant 1508 is seen in the ultrastructure by scanning and transmission electron microscopy (SEM and TEM, respectively). Mutant 1508 is almost completely devoid of small granules when observed by SEM, indicating that the formation of both the hordein-containing protein bodies and the small starch granules are affected (I). As it is very difficult to identify small spheres observed in the scanning electron microscope (Munck 1972; I) an estimate of the reduction of either small granules, protein bodies or small starch granules could not be achieved. TEM studies display, however, that the composition of the protein bodies of mutant 1508 is different from Bomi (Ingversen 1975; Munck and Wettstein 1976). The protein bodies of the mutant contain, as a dominating component, a granular matrix in which small homogenous globules are embedded, in contrast to Bomi, in which the homogenous structure is dominating and surrounded by the granular network. These changes in protein body structure in the mutant are suggested to reflect the qualitative as well as the quantitative alterations in hordein biosynthesis caused by the lys3a gene mutation (Cameron-Mills 1980). The deficiency of small starch granules in mutant 1508 is suggested by Mifflin and Shewry (1979b) to be caused by some biochemical lesions of starch synthesis in certain amyloplasts. The reduction of the number of small starch particles does not, however, account for the total 20% reduction of starch in the mu-

tant compared to Bomi (Kreis 1979).

All the characters described above affecting the seed metabolism of mutant 1508, in particular the reduced starch content, have resulted in a shrunken seed. The reduced grain yield of the mutant compared to Bomi is thus mainly attributed to the reduced seed size because of the shrunken state (Doll et al. 1974). Mutant 1508 has been included in many breeding programs, but so far the impossibility of breaking the relationship between high lysine content and shrunken endosperm makes it unsuitable for practical breeding work.

Mutant 7 represents a very modest lysine increase of protein, 10% compared to the mother variety Bomi, most probably caused by a single recessive gene inheritance (Doll 1976). The chromosomal location of the gene is at present studied at Risø (Doll pers. commun.). This high-lysine barley mutant is now included in many breeding programs because it is so far the only one found without reduction in seed size in comparison to its mother variety. The grain yield has been reported to be normal in the USA (Stobart, cf. Kjøie and Doll 1979) but both field and pot experiments at Risø show a decrease of 10% mainly due to a reduced seed number compared to Bomi (Kjøie and Doll 1979).

The mutant 7 gene acts in a similar way as the lys3a gene in mutant 1508 in altering the protein composition. Compared to normal barley, the reduction of the hordein proteins in mutant 7 is thus compensated for by a concomitant increase of non-hordein proteins, including a slight increase of non-protein nitrogen, but not to the same extent as in mutant 1508 (Kjøie and Doll 1979; IV). Like mutant 1508 the glutelin content of mutant 7 is enhanced in per cent of total protein but the total amount of glutelin per seed is much higher (V).

Apart from the great lysine contribution of the residual fraction in mutant 7, most of the lysine comes from the glutelin fraction. Despite an increased lysine content of the hordein proteins, they constitute only a very small part of the total amount (V). Anyhow, the polypeptide composition of the hordeins in mutant 7 is clearly changed from that in Bomi, which is evident from electrophoretic separation of these proteins (Doll pers. commun.).

The starch content of the mutant 7 seed is only slightly decreased com-

pared to Bomi and with no increase in soluble sugars as in mutant 1508 (Kjøie and Doll 1979).

The changes in seed metabolism by the mutant 7 gene is not as drastic as by the lys3a gene in mutant 1508. It seems the genes are acting in a similar way with different degrees of expression. There is some indication that the genes are influencing the biochemical pathways differently, which applies especially to the highly increased total amount of glutelin proteins of mutant 7 compared to mutant 1508.

Besides these two high-lysine barley mutants, most of the other Risø mutants follow type A1 for improvement of the lysine content in the seed. This is also valid for the Notch mutants described by Balaravi et al. (1976) and the lys 95 and lys 449 mutants described by Di Fonzo and Stanca (1977). The high-lysine character of the Risø mutants and Hiproly is controlled by single genes (Munck et al. 1970; Doll 1973, 1976; Kjøie et al. 1976) while comparable genetic studies have not been reported for the Indian and Italian mutants. A summary of the origin of the A1 high-lysine barley genotypes in addition to those described as A2 is out-lined in Table 2.

It is observed in Table 2 that the high-lysine genes are distributed over almost the whole barley genome. All the genes are characterized by a recessive inheritance except the Lys4d gene in mutant 8 which is dominant (Jensen 1979). The Hor2ca gene in mutant 56 is recessive in its qualitative effect on the hordein polypeptide composition and co-dominant with respect to the relative amount of hordeins (Doll 1980). The lysine increase of mutants 13, 29 and 86 is controlled by alleles of the gene sex1a on chromosome no.5 (Jarvi and Eslick 1975; Ullrich and Eslick 1978; Jensen 1979) while the lys and lys3a gene on chromosome 7 are non-allelic (I) and independently inherited (Muench et al. 1976). The somewhat confusing gene designation of the high-lysine barley genotypes, discussed by Jensen and Doll (1979), might hopefully be brought into order in the future.

Table 2. Origin of high-lysine barley genotypes

Type	Genotype	Derived from	Gene localized on chrom. no.	Gene	Ref.
A1	29	Carlsberg II	6	<u>sex1a</u>	1,2
"	86	Carlsberg II	6	<u>sex1a</u>	1,2
"	7	Bomi	not localized	-	3
"	8	Bomi	5	<u>Lys4d</u>	4
"	13	Bomi	6	<u>sex1a</u>	5
"	16	Bomi	1	-	5
"	17	Bomi	1	-	5
"	527	Bomi	6	-	5
"	1508	Bomi	7	<u>lys3a</u>	5,6
"	Notch-1	NP 113	not localized	-	7
"	Notch-2	NP 113	not localized	-	7
"	<u>lys</u> 95	Perga	not localized	-	8
"	<u>lys</u> 449	Perga	not localized	-	8
A2	Hiproly	World Barley Collection	7	<u>lys</u>	9
"	56	Carlsberg II	5	<u>Hor2ca</u>	10

Ref.1. Jarvi & Eslick (1975), 2. Ullrich & Eslick (1978), 3. Doll (1976), 4. Jensen & Doll (1979), 5. Jensen (1979), 6. Karlsson (1977), 7. Balaravi et al. (1976), 8. DiFonzo & Stanca (1977), 9. Karlsson (1976), 10. Doll (1980).

A2 - increase of specific lysine-rich proteins

Lysine improvement in the barley seed can also be caused by an increase of certain lysine-rich "Osborne" protein fractions without a decrease of the storage protein fraction or/and an increase of lysine-rich components of a specific protein fraction. Hiproly barley is such an example whereas mutant 56 has some properties according to this type but others according to type A1.

Hiproly is the only high-lysine barley genotype in which the improved lysine level is not caused by mutagenic treatment, being a spontaneous mutation found in the World Barley Collection (Munck et al. 1970). In this variety the lysine as well as the protein content is raised with 30% compared to normal-lysine barley, differing thus from the Risø mutants, which display only minor increases in protein content. When crossed with commercial cultivars the high-protein character in the high-lysine lines is, however, more or less lost (Persson and Karlsson 1977), demonstrating that the high-lysine and high-protein characters are independently inherited. The increased lysine content of Hiproly, which is due to the single recessive lys gene expressed in the endosperm, is generally considered to be caused by an increase of the water- and salt-soluble proteins in addition to the glutelin proteins, whereas the hordeins are only slightly reduced and the nonprotein nitrogen content is unchanged compared to normal-lysine barley.

Considering the hordein content in Hiproly and derived high-lysine lines, some data indicate almost no reduction compared to normal barley (Berdahl and Bhatti 1977; El-Negoumy et al. 1977; IV), others indicate a slightly higher reduction (Munck 1972; Miflin and Shewry 1979b; V). These varying results, which depend on different extraction methods and the normal-lysine type used as a standard, are summarized by Miflin and Shewry (1979b). The divergence between the data for a Hiproly back-cross line (IV, V) is probably caused by contamination with normal barley in the first case, which also explains the low total lysine content of seed protein (IV). It is therefore concluded that Hiproly and the Risø mutants as well as the Indian and the Italian mutants are characterized by a reduction of hordein but not to such a degree that Hiproly could be considered a "low-hordein type" and follow type A1.

The highly increased lysine percentage of the water- och salt-soluble proteins of Hiproly and high-lysine lines (Munck 1972; Berdahl and Bhatti 1977; El-Negoumy et al. 1977; Jonassen 1980; V) makes this high-lysine variety unique, compared to all the other high-lysine types which are characterized by an almost unchanged lysine content of the water- and salt-soluble proteins (Shewry et al. 1980b).

Four major salt-soluble proteins i.e. β -amylase, protein Z, chymotrypsin inhibitor CI-1 and CI-2 are contributing to this lysine enhancement and account for more than 50% of the increased seed lysine,

which corresponds to 17% of total seed lysine from Hiproly lines compared to 7% from normal barley (Hejgaard and Boisen 1980). Most of the lysine contribution from these four proteins comes from protein Z, which has extraction properties similar to β -amylase, with which it forms aggregates. Compared to normal barley, protein Z displays a 2-fold increase while the amount of protease inhibitors per g seed is 7 times higher in Hiproly lines. In a parallel study Jonassen (1980) has isolated two albumin proteins SP II A and SP II B, which account for 37% of the lysine increase in Hiproly compared to normal lysine barley. The SP II proteins have later been immunochemically identified as chymotrypsin inhibitors CI-2b and CI-2c, respectively (Svendsen et al. 1980). Despite the highly increased lysine content of the water-soluble fraction most of the total lysine contribution in Hiproly as well as in mutants 7 and 1508 (V; Tallberg, unpubl.) comes from the glutelin fraction, although the increase of lysine in Hiproly-lines is mainly caused by the increased amounts of the four lysine-rich components of the water- and salt-soluble fraction. These proteins may be regarded as storage proteins, as they exert storage features i.e. the inhibitors are accumulated during endosperm development and disappear during germination, and their amount as well as the amount of β -amylase and Z-protein increases with nitrogen fertilization (Kirsi 1973; Hejgaard and Boisen 1980).

The problem of shrunken endosperm of Hiproly has been easily overcome by back-crossing to high-yielding varieties (Persson and Karlsson 1977), but the grain weight and, thus, the grain yield of the high-lysine lines are not as high as in the standard. The starch content in absolute figures is less, whereas the percentage of starch of total grain weight is the same or even slightly higher in the high-lysine lines compared to their normal-lysine parent varieties (Hagberg et al. 1979). It seems, however, that the rate of the grain growth in the high-lysine lines has been slowed down in comparison to their normal counterparts. These results cannot entirely explain the smaller seed size of the Hiproly-lines, but investigations regarding phytohormones regulating the seed size might be elucidating (Mounla et al. 1980). The effects of the lys gene in contrast to the lys3a gene have not influenced total seed metabolism so drastically, which makes Hiproly the most promising high-lysine barley in breeding for improved protein quality.

Mutant 56, derived from the commercial cultivar Carlsberg II, has an enhanced lysine level of 17% in the seed protein (Doll 1976). This high-

lysine barley genotype is suffering from a reduced seed size as well as a reduced number of seeds compared to Carlsberg II (Køie and Doll 1979). The 10% lower grain weight partly reflects a reduced starch content accompanied by an increased content of soluble sugars.

The gene Hor2ca, responsible for this increased lysine content, is located at or near the Hor 2 locus on chromosome 5, containing the structural genes for the hordein-2 proteins, and is not linked to the translocation between chromosomes 2 and 5 in the mutant (Doll 1980). The mutation has strongly influenced the hordein content by reducing the hordein-2 and increasing the hordein-1 proteins (Køie and Doll 1979) with a net hordein reduction of 25% of total protein compared to Carlsberg II (Mifflin and Shewry 1979b; Køie and Doll 1979). The reduction of hordein-2 proteins is seen in micrographs of isolated protein bodies showing fewer homogenous components embedded in a larger proportion of fibrillar matrix, compared to Carlsberg II (Cameron-Mills 1980). The decreased hordein content is compensated for by an increase of nonprotein nitrogen as well as salt-soluble and glutelin proteins. According to Shewry et al. (1980b) the main effects on protein composition in mutant 56 are observed in increased total amounts of nonprotein nitrogen and glutelins.

Preliminary results (Hejgaard pers. commun.) indicate that the four water-soluble protein components in Hiproly, are also increased in mutant 56. To what extent this increase contributes to the high lysine level must be determined by further studies. So far only one value for the lysine content in the salt-soluble fraction is reported (Shewry et al. 1980b) and this is not higher in mutant 56 compared to Carlsberg II. This is different from Hiproly, in which the increase of the four salt-soluble storage proteins corresponds to a total lysine increase in the water-soluble fraction (Hejgaard and Boisen 1980). The unchanged lysine content of the hordein fraction in mutant 56 compared to Carlsberg II (Shewry et al. 1980b) suggests, however, a similarity to Hiproly. Before the causes of the lysine increase in mutant 56 are entirely established, I prefer to put this mutant under the heading of type A2. This is based on the fact that, despite the rather high hordein reduction, mutant 56 is the only mutant, besides Hiproly, yet reported to have increased amounts of the salt-soluble storage proteins.

Summarizing the characterization of the four high-lysine barley genotypes described, it is evident that they represent a wide range of changes with regard to protein quality as well as genetic background. The percentage of hordein and starch decreases in order Hiproly, mutant 7, mutant 56, and mutant 1508, which reflects the decreasing grain yields. This concomitant impairment of hordein and starch synthesis in the endosperm indicates a possible interrelation between the principal storage products, which should be further studied in order to understand the grain-filling mechanism of the high-lysine barley genotypes.

The enhanced lysine content of both mutant 7 and 1508 is caused by the increase of non-hordein proteins resulting from a reduced hordein synthesis. These two mutants, in which the lysine improvement is due to the protein alterations, could thus be termed "low-hordein mutants". Mutant 56, with a pronounced hordein reduction, and Hiproly are both characterized by an increased content of certain lysine-rich salt-soluble proteins with storage features, but only Hiproly has a higher lysine content in the salt-soluble fraction, compared to normal-lysine barley. As the lysine improvement of Hiproly is mainly caused by the increase of these salt-soluble storage proteins with only a slight reduction of hordeins, Hiproly should not be considered a "low-hordein type". Whether mutant 56 should be termed a "low-hordein mutant" depends on how much the salt-soluble storage proteins contribute to the lysine increase in this mutant. Although the four high-lysine barley genotypes have lysine improvement of different origin, it is the glutelin proteins that contribute with the highest lysine percentage of total seed lysine.

A3 - *increase of lysine in the lysine-poor prolamins*

According to the definition of prolamins (Mifflin 1978) these proteins exhibit a storage function and are very poor in lysine. A tentative way for lysine improvement in barley seeds could thus be to increase the lysine content of the hordein proteins, as a manipulation of these storage proteins would probably not affect the seed metabolism.

Mutant 1508 (Shewry et al. 1980) and mutant 7 (V) are both characterized by an increased lysine content of the hordein proteins. Despite this

raised lysine level the hordeins contribute only marginally (5-10%) to total seed lysine. This depends both on the reduced hordein content of these mutants and the very low amount of lysine in this fraction (V). As calculated in (V) the lysine content of hordeins should be raised with 200% in order to significantly affect total lysine at an unchanged content of hordein in the seed protein. Suggestions to evaluate a possible variation of the lysine content of the hordein polypeptides (Blake 1981) in order to combine potential lysine-rich ones for obtaining a total lysine increase in the seed, seem therefore unconvincing. Furthermore, hordein is a multigene family of structurally related proteins (Faulks et al. 1981; Shewry et al. 1981) which makes it difficult to accumulate sufficient mutational changes in the hordein genes to affect significantly the overall amino acid composition of the barley seed. One may also hypothesize whether increased amounts of lysine in the hordein proteins could change their hydrophobic character in the direction that they cannot precipitate in the protein bodies and thus would not be recognized as hordeins because of the changed solubility properties.

So far, where a raised lysine level of the hordein fraction has been obtained, it is accompanied by a reduction of the hordein content. A reduction of hordein proteins is thus more important than a higher lysine concentration for lysine improvement of the seed protein.

The concomitant reduction of starch synthesis recognized in the high-lysine barley mutants with a reduced hordein content is unfavourable to the grain yield. The apparent interrelation between hordein and starch synthesis indicates an unaffected hordein accumulation of significance for an efficient grain filling. As proposed by Doll (1981) the most promising breeding strategy for lysine improvement in the barley seed may, hence, be to replace the hordein by more lysine-rich storage proteins, as is the case in Hiproly.

Lysine improvement in barley seed protein through increased amounts of lysine in the hordein proteins seems at the moment most unthinkable.

B1 - increase of embryo/endosperm weight ratio

In the cereals the embryo proteins are much superior in lysine content to proteins of the endosperm, as well as, in other essential amino acids

required by man and monogastric animals. If a higher proportion of the seed protein were embryo protein, the protein quality would be increased. An increased embryo/endosperm weight ratio of barley seeds might thus increase the lysine content. A comparison of embryo/endosperm ratios for high- and low-protein barley cultivars and Hiproly and the effect on lysine contribution from the embryo showed no significant differences (Munck 1970). It was therefore concluded that the increased level of lysine in Hiproly, due to the lys gene, is caused by major protein alterations in the endosperm.

Mutant 1508 exhibits a larger embryo both relatively and in absolute value compared to Bomi (II). Dry embryo weight at dead ripeness is in mutant 1508 2.8 mg and in Bomi 2.0 mg. This corresponds to a relative embryo size of 6.9 in mutant 1508 compared to 3.6 in Bomi. Despite this highly increased embryo/endosperm weight ratio in mutant 1508, the embryo lysine of Bomi contributes as much to the total lysine content of the seed as the embryo lysine of mutant 1508. It is likely that the lysine contribution to total seed lysine from the larger embryo of mutant 1508 would be more pronounced if the formation of starch and storage proteins in the endosperm had been unaffected. The amino acid pattern of the embryo is almost the same in mutant 1508 as in Bomi, although the amino acid composition of the mutant endosperm is drastically changed. It is shown that the difference between mutant 1508 and Bomi in overall amino acid composition, particularly in lysine, can be attributed entirely to the differences in protein composition of the endosperm (II).

As the embryo in barley accounts for only 3-5% of the whole seed which corresponds to c. 15% of total seed lysine an increased embryo/endosperm weight ratio as in mutant 1508 (7%) influences the total seed lysine only marginally. Hitherto no high-lysine barley genotype has been found, in which the lysine increase can be attributed to an increased embryo/endosperm weight ratio.

B2 - *increased number of aleurone layers*

Another morphological change of the cereal seed that might increase the lysine content is a multilayered aleurone. This part of the cereal seed is rich in water- and salt-soluble proteins and hence in lysine. Yet, no high-lysine barley genotype has been found with a multiple al-

aleurone layer but Hiproly displays a larger subaleurone layer compared to normal barley (Munck et al. 1970; Munck 1972; Bechtel and Pomeranz 1979). The subaleurone layer seems to contribute in a minor degree to the total lysine content, as the normal-lysine, near iso-genic Sisterline (CI 4362) has this layer equally well-developed as has Hiproly. A higher protein content in the subaleurone tissue is observed in maize (Nelson and Chang 1974). If this is true for barley as well, the higher percentage of seed protein in Hiproly and its Sisterline could be partly explained.

A small but definite enhancement of the lysine content in high-lysine opaque-2 maize by incorporation of a gene conditioning multiple aleurone layer suggests that multiple layering may be advantageously included in breeding programs (Nelson and Chang 1974). This may not be valid for the barley seed, as the maize seed displays a single cell aleurone layer whereas barley has three cell layers. Whether a lysine increase caused by multiple aleurone layers in barley is a nutritional improvement can be questioned, as digestible energy and biological value decreases with an increased amount of aleurone cells (Bach Knudsen 1980).

C - *interactive effect of combined high-lysine factors*

Combining two different high-lysine factors is the third major possibility for improvement of lysine content in the barley seed. The high-lysine segregants containing both factors are, in the following, called "double-recessives" (IV, V), as the genes responsible for the increased lysine content are recessive in their inheritance.

The lysine content in the double-recessives from the combination between the lys3a and the lys genes corresponding to mutant 1508 itself and a Hiproly back-cross is increased with c. 15% with a value of 5.5g/16gN compared to 4.75g/16gN in mutant 1508 (IV). The lysine content in the double-recessives compared with the other parent (Hiproly x Mona⁵) is increased by 50%. The lysine value reported for Hiproly x Mona⁵ (3.66g/16gN) is lower than expected, depending on a possible contamination of normal barley in this very sample, which could explain the high lysine increase in the double-recessives compared to the Hiproly back-cross.

The increased lysine content of the double-recessives depends on pro-

tein alterations in the endosperm. The almost completely suppressed hordein synthesis is replaced by an enhancement of free lysine as well as the lysine-rich water-soluble and glutelin proteins. The double-recessives follow the same pattern as mutant 1508 but in a more drastic way, the hordeins being decreased with 40% while the content of free lysine, the water-soluble and glutelin fraction is raised with 30, 20 and 35%, respectively, compared to mutant 1508. The lysine distribution in the different protein fractions follows, however, the Hiproly-pattern (Tallberg, unpubl.). The double-recessives are thus characterized by almost as much lysine in the water-soluble fraction as reported for Hiproly (Munck 1972; Berdahl and Bhatti 1977; El-Negoumy et al. 1977; Jonassen 1980; V), which is a clear distinction from mutant 1508. It is obvious that both high-lysine genes, lys and lys3a, are phenotypically expressed in the lysine increase of the double-recessive seeds in an interactive way.

The seeds of the lys3a/lys double-recessives are extremely shrunken with a decrease in grain weight at yellow ripeness of 30% compared to mutant 1508. The starch content of the double-recessives is, however, the same as in mutant 1508 (Tallberg, unpubl.) which means that the reduced grain weight cannot be ascribed to a further impaired starch synthesis compared to mutant 1508.

As discussed by Kreis and Doll (1980) the resemblance of two high-lysine genes with respect to the way they influence the protein and starch synthesis can be elucidated by their interaction in segregants containing both genes. If the same pathway is affected, the double-recessive is expected to have the same value as the most deviating gene in a single dose. The very fact that the lys3a/lys double-recessives have the same content of starch as that conditioned by the single lys3a gene, indicates that the lys3a and the lys gene influences the same pathway in starch synthesis. The total reduction of hordein is, however, much greater than the reduction caused by each single gene. In this case the lys3a gene is acting in an additive way together with the lys gene, which indicates that the lys3a gene influences hordein synthesis in a different way than the lys gene.

The major changes in the seed metabolism affecting proteins and carbohydrates indicate that the possibilities of increasing the grain weight

of the lys3a/lys double-recessives may be limited. By transferring the double-recessives into new genetic back-grounds the agronomic characters might be improved.

To combine two high-lysine factors for obtaining an increased lysine content in the barley seed, but with no extreme reduction of grain weight, mutant 7 was crossed with the same Hiproly back-cross line as was mutant 1508. The reasons are unknown for the observed heterogeneity with regard to the lysine content in the seed protein of the double-recessives. Therefore, mean values will be used in the proceeding summary of (V).

The lysine content of seed protein is increased with c. 15% in the double-recessives compared to either high-lysine parent, giving a total value of 4.6g/16gN. This sample of the Hiproly back-cross compared to a standard is not contaminated by normal barley, and a higher lysine value is therefore obtained.

Regarding the protein distribution in the different "Osborne" fractions, the 7/lys double-recessives are characterized by a reduced and almost unchanged hordein content compared to the back-cross line and mutant 7, respectively. In the water-soluble and glutelin fraction the double-recessives exhibit similar values compared to Hiproly x Mona⁵ and higher than that for mutant 7. In mutant 7 the total amount of glutelins is higher, which is also reflected in the double-recessives compared to the Hiproly back-cross. The 7/lys double-recessives have a higher lysine content in the water-soluble protein fraction than even the Hiproly back-cross, whereas the lysine content in the hordein fraction is almost the same as in mutant 7, but definitely higher than in the back-cross line. It is concluded that the lys and mutant 7 genes have an interactive effect on the lysine increase in the double-recessives. It can also be stated that the genes influence the hordein synthesis in different ways, as the total hordein reduction caused by the combination of the genes is greater than the reduction observed for each individual gene.

The overall impression of the 7/lys double-recessives is that their changes in the seed metabolism are modest compared to the lys3a/lys double-recessive. Besides the modest protein alterations in the endosperm, the double-recessives are characterized by only a 5% reduction of available carbohydrates, e.g., starch and free sugar (VI). The starch content in the 7/lys double-recessives, compared to the Hiproly back-cross and mutant 7, is un-

changed and slightly lower, respectively (Tallberg, unpubl.). It could be anticipated that the lys and mutant 7 genes influence the starch synthesis pathway in a similar way, but further studies will be needed.

The grain weight of the 7/lys double-recessives is 20% lower compared to normal lysine barley but in contrast to the lys3a/lys double-recessives the seeds are not shrunken. For practical breeding purposes the 7/lys double-recessives therefore seem promising.

So far, mutant 7 is the only high-lysine barley mutant in which the glutelin synthesis is enhanced. The effect of this variation on amino acid composition is probably not large in itself, but in the double-recessive combination with the lys gene a more favourable amino acid composition is produced (VI) without a drastic deficiency of storage proteins. The glutelin proteins constituting the second major class of proteins in the barley seed are almost always increased in the seed protein of the high-lysine mutants and, particularly, of the different double-recessives. The great proportion of the glutelin fraction in combination with its high lysine content results in the highest lysine contribution from these proteins compared to the other proteins in the seeds of high-lysine barley as well as normal-lysine barley. Too little attention has, however, been paid to the glutelin proteins in evaluating the protein quality of high-lysine barley genotypes. This might be explained by insufficient protein extraction methods causing hordein contamination of the glutelin fraction and, hence, masking the true functions of the glutelins. As suggested by Mifflin and Shewry (1979a) the glutelins are unlikely to be storage proteins and are probably membrane proteins constituting the "matrix" proteins of the cell. The uncertainty regarding the functions as well as the localization of the glutelins in the cell thus calls for further investigations into the improved protein quality in barley seeds.

LYSINE-SCREENING METHODS

In selecting mutants or lines with deviating lysine content either for screening or for breeding purpose it is essential that the method used is reliable, rapid and cheap. The screening method should demand a minimum sample and preferably be used on a single seed basis. Amino acid

analysis by ion exchange chromatography gives the most accurate estimation of the amount of lysine but it is too slow and expensive. For large-scale screening programs this method is unsuitable, but for checking the lysine amount in samples selected by a less exact method it is invaluable. The different screening methods for lysine-deviating samples can be divided into two groups, those detecting the lysine increase directly and those indicating the lysine increase by measuring other properties.

The DBC/N method

Several methods for estimating lysine directly in cereal seeds have been reported in the literature but few of them are routinely used. Three methods were compared with regard to the reliability and validity of the lysine estimation in a study (III) of a barley material with varying amino acid composition. The dye-binding capacity method - DBC - has been very successfully employed at Svalöf, Sweden, and Risø, Denmark, for identification of high-lysine barley strains and mutants (Munck 1976; III). Hiproly with a 30% lysine increase (Munck et al. 1970) as well as mutant 1508 and mutant 7 with 45 and 10% lysine increase, respectively (Doll et al. 1974), were identified by means of this screening method. Here, the amount of Acilane Orange G dye bound to the basic imidazol-, guanidine- and amino groups corresponding to the basic amino acids histidine, arginine and lysine, respectively, is compared with the nitrogen content of barley seed meal. The high mutual correlations between the individual basic amino acids (III) point to the applicability of the DBC/N method for lysine screening. In practice, DBC and N values are plotted in a graph and any sample with a higher DBC/N ratio and, thus, deviating from the regression line is likely to have an increased amount of lysine. In the DBC method proteins are precipitated at an acid pH and bound to the dye, the result being a recorded decolorization of the supernatant (Udy 1954, 1956). As mutant 1508 and the 1508 double-recessives contain large amounts of free amino acids in per cent of nitrogen content (Brandt 1975; IV) a reduced precipitation of dye on a given N-level will occur and consequently a lower DBC value will be obtained. This explains why the double-recessives, despite a higher lysine value, cannot be selected from the 1508 segregants by the DBC/N method (IV). Although mutant 1508 itself displays a lower DBC value, this deviation in dye-binding does not influence the selection because of the high total amount of dye bound by this mutant compared to normal-lysine samples.

The DNP method used at CIMMYT

An alternative lysine screening method which is used with success in high-lysine maize breeding at CIMMYT, Mexico, makes use of 2-chloro-3, 5-dinitropyridine (DNP) as a reagent (Villegas and Mertz 1971). After partial enzymatic hydrolysis of the proteins in the meal suspension and blocking the α -amino groups of the free amino acids with copper, ξ -dinitropyridyl-lysine is formed and measured spectrophotometrically (Tsai et al. 1972). As reported (III) the DNP analysis is equally well suited for determining lysine in barley as in maize. The DNP method like the DBC method is probably measuring the amount of basic amino acids (BAA) rather than lysine alone as the correlation between DNP and BAA as well as DNP and DBC is significantly high. Despite the large differences with regard to the chemical reactions of the DBC- and DNP methods, both are equally reliable for selection of high-lysine deviates in barley. In practice the DBC method is easier to perform and as the DNP analysis is mainly used at CIMMYT as a further analysis after tryptophan screening with the same hydrolysate it is concluded that for lysine screening in barley DBC/N is a more appropriate method.

A fluorescence method based upon dansylation

In the third lysine predicting method compared (III) the dansyl-derivate of lysine is determined by fluorescence (Christoffers et al. 1974). This method is, however, not as valid as DBC and DNP for lysine estimation, the correlation to lysine determined by ion exchange chromatography being lower than for DBC and DNP. The observation that the fluorescence analysis fails to distinguish mutant 1508 from normal lysine samples (Køie pers. commun.) makes this method unsuitable for lysine screening of a barley material with a wide variation in amino acid composition.

In preliminary screening work, when it is not necessary to determine a lysine value but only to detect aberrants, it is desirable that the methods can be performed on a single-seed basis. Such methods are often based on measurement of a phenotypic character associated with a known high-lysine gene or distinguished by several high-lysine genes.

The "turbidity-test"

Since all the high-lysine barley mutants have a reduced hordein content

it is likely that a hordein-estimating method can be used as a lysine-screening method as well. The "turbidity-test", developed for selecting mutant 1508-segregants (Køie and Nielsen 1977) is based on precipitation of hordeins from a single seed in an alcohol solution with water. As normal hordein samples display a heavy precipitation while mutant 1508 and other low-hordein mutants do not, the method clearly identifies samples with a low hordein and, hence, a high lysine content. The analysis is advantageously employed in a large-scale screening program at Risø, Denmark, for identification of low-hordein aberrants which can be further used in studies of gene control of storage protein synthesis (Doll pers. commun.). On the other hand, the method is disadvantageous for selecting high-lysine genotypes with an increased or novel synthesis of other lysine-rich proteins with no or with only a slightly reduced hordein synthesis, e.g., Hiproly.

Determination of amide N in per cent of total N

The amount of amide N expressed as a percentage of total seed N has been used as a selection criterion of high-lysine barley genotypes because of a strong negative correlation between % amide N and % lysine (Toft-Viuf 1972). Since a low percentage of amide N in the seed is related to a low hordein content (Kirkman et al. 1981) samples with low hordein and presumably high lysine content is selected. This selection procedure suffers, consequently, from the same disadvantage as the "turbidity-test" in not detecting high-lysine normal-hordein genotypes. However, as far as I know, this method is not applied anywhere for selection of high-lysine barley deviants.

The selection criterion mentioned above is based on a character — low hordein content — which is common for all high-lysine barley mutants described. Other methods depend on a specific character associated with only one high-lysine gene. As the lysine increase due to the lys gene depends on enhanced amounts of β -amylase, Z-protein and two chymotrypsin inhibitors (Hejgaard and Boisen 1980) it seems reasonable to screen Hiproly-derived lines for an increase of these proteins, which contribute significantly to the total increase of lysine in the seed.

Enzymatic determination of β -amylase and chymotrypsin inhibitors

β -amylase activity, closely associated with the high-lysine character

of Hiproly and 4-6 times higher in the high-lysine segregants irrespective of the normal-lysine parent variety in the cross-breeding, shows a high correspondence to the DBC/N method (Hejgaard et al. 1979). The separation between the high- and normal-lysine groups is, however, not as clear-cut as when the activity of the chymotrypsin inhibitors is measured, which indicate a higher validity for the enzymatic inhibitor activity assay (Hejgaard and Boisen 1980). This latter method is reported to be highly correlated to DBC/N when used for single seed analysis (Hejgaard and Boisen 1980). The correspondence is, however, not sufficiently established when used on a Hiproly barley material in which the genetic back-ground is very wide (Tallberg, unpubl.).

Radial immunodiffusion

An alternative method for estimating the protease inhibitor activity is based upon an immunological reaction between a compound of the SP II proteins and antibodies raised against it (Jonassen 1980). This single radial immunodiffusion technique can also be carried out by single seed analysis (Jonassen 1980) and preliminary unpublished data from studies with a population segregating for the lys gene, display a complete correspondence to DBC/N. The immunodiffusion assay is not as laborious as the enzymatic inhibitor assay. Because the validity seems higher for the first method, this assay might become instrumental in preliminary screening for the lys gene in large-scale breeding programs.

Electrophoresis

Electrophoresis of different protein fractions can also be carried out for screening samples with deviant protein composition. A large-scale screening program by polyacrylamide gel electrophoresis of hordein proteins is thus performed on M_2 -seeds from a sodium acid treated material at Carlsberg Research Center, Denmark, for identification of samples having changed hordein polypeptide composition. The selected aberrants are used for studies of gene control of hordeins and in a wider sense, gene control in eucaryotic cells (Ingversen pers. commun.).

In extensive breeding programs for high-lysine barley it is of importance that the screening work can start in an early generation. This is achieved by methods based on single-seed analysis. Unfortunately, there is yet no method for direct estimation of the lysine amount in a single seed, except

ion exchange chromatography. When a known high-lysine gene is put into cross-breeding, the breeder can, however, in preliminary screening depend on indirect lysine selection methods, which measure the lysine-increasing characters expressed by the gene. The high protease inhibitor activity in Hiproly-derived lines, which is due to the lys gene, is such a lysine-increasing character that might easily be selected for by means of radial immunodiffusion.

When an unknown barley material is screened for increased lysine content, the method used must indicate the lysine increase. The DBC/N method has hitherto been most successful, Hiproly and all high-lysine mutants found at Risø, Denmark, being selected by this method. The considerable progress achieved at Svalöf in high-lysine barley breeding (Persson and Karlsson 1977) depends entirely on DBC/N for lysine screening, though some selected samples were checked by ion exchange chromatography.

BIOLOGICAL EXPERIMENTS - FEEDING TRIALS

When a high-lysine genotype is identified and subsequently introduced into a breeding program, it is of great importance that the nutritional quality is determined not only by chemical analyses but also by biological experiments with rats, poultry or pigs.

In rat nitrogen balance tests true digestibility (TD), biological value (BV) and net protein utilization (NPU) — parametres measuring the nutritional value — are improved in Hiproly compared to normal-lysine barley (Munck et al. 1970). Also in work with broilers (Elwinger 1973) and pigs (Thomke and Widströmer 1975) the superiority of Hiproly barley is observed. Rat nitrogen balance tests show that the very high lysine content of mutant 1508 leads to an almost 20% increase in BV compared to Bomi (Eggum 1978). TD is, however, reduced for mutant 1508 resulting in only a 10% increase of NPU compared to Bomi. On an average, the different high-lysine barley mutants hitherto analysed with rat nitrogen balance test display higher values for BV although both higher and lower values for TD and consequently NPU are obtained (Eggum 1978).

The chemical analyses of the lys3a/lys and 7/lys double-recessives indicate an improved nutritional quality of the protein. As the glutelin proteins form the major protein class of total seed protein in these

double-recessives, they might also influence the nutritional value. These proteins, forming large aggregates linked by S-S-bridges, with high molecular weights, are difficult to extract and hence it could be expected that they are difficult to digest, thereby affecting the nutritional value in a negative way. Nevertheless, the nutritional quality of the double-recessives is improved as rat nitrogen balance tests show that BV, NPU and UP (utilizable protein) are significantly higher although TD is only slightly and insignificantly lower in the 7/lys double-recessives compared to normal-lysine barley (VI). In comparison to mutant 7 all four parameters constituting the nutritional quality are increased in the 7/lys double-recessives.

The lys3a/lys double-recessive also shows significantly increased values for BV, NPU and UP while TD is significantly decreased compared to normal lysine barley (VI). As pointed out by Bach Knudsen (1980) the digestibility problems with mutant 1508 are entirely confined to the endosperm portion of the seed and thus the highly suppressed hordein synthesis in the double-recessive as well as in mutant 1508 might partly explain their lower protein digestibility. The deviating values obtained in this study for the Hiproly back-cross compared to those for Hiproly itself (Munck et al. 1970) are probably caused by an unpure back-cross line, as described earlier.

The enhanced level of lysine and other essential amino acids (in particular threonine, usually the second limiting amino acid in cereal grain diets) in the 7/lys double-recessives has significantly increased the biological value. This improvement is undoubtedly of importance to meet the essential amino acid requirement of man as well as monogastric animals. The present work has clearly demonstrated that it is possible genetically to upgrade the nutritional quality of barley protein.

YIELDING PROBLEMS

It is observed that all high-lysine barley genotypes hitherto identified suffer from a reduction of grain yield relative to standard. The grain yields of the Risø mutants are reduced generally by a combination of fewer and smaller seeds, and the reduced grain weight is the major reason for the low yields (Kjølle and Doll 1979). Mutant 1508 with the very high lysine content has been included in several breeding programs all over

the world, but selections tested for grain yield and seed characters have not surpassed those of the mutant. The shrunken seed associated with the high lysine content in mutant 1508 has so far been impossible to get rid of, probably as a consequence of the drastic alterations in seed metabolism that affect the mutant. Since the lysine content of mutant 1508 is extremely high, total lysine yield is highly improved compared to standard (Persson and Karlsson 1977). This advantage of mutant 1508 is, however, cancelled when considering its very low energy availability, which is economically detrimental in animal production (Bach Knudsen and Munck 1981).

The yield of Hiproly itself is about one third of the yield of conventional barley cultivars. Through back-crossing, the yield level has been raised to 85-95% of the recurrent parent (Persson and Karlsson 1977). The yield reduction depends on the small seed size of the high-lysine lines, and so far the negative correlation between seed size and lysine content has been impossible to break (Persson and Karlsson 1977). The reasons for the small seed size are not clarified but the slightly lower amount of hordein as well as starch might be of significance. Nevertheless, most progress and hence hope for high-lysine barley breeding is devoted to Hiproly. The potential value of high-lysine barley for animal production, using Hiproly as the lysine source, has been confirmed in pig feed trials, in which protein supplementation could be reduced (Thomke et al. 1978). High-lysine barley for human consumption is hopefully not far away, since Hiproly-lines are incorporated and adapted in national breeding programs in several developing countries.

Yield, however, must not be sacrificed when incorporating the high-lysine characters into well-adapted barley cultivars. To what extent can the yield deficiencies of the high-lysine barley variants be overcome by breeding efforts? Considerable progress might be made by selecting for genetic back-grounds that interact harmoniously with the high-lysine genes. Favourable modifier genes known to be associated with the lys gene can play an important role in influencing the grain weight and, thus, the yield. Through accumulation of favourable modifiers, as in high-lysine opaque-2 maize (Vasal et al. 1979), the yield gap between high-lysine and normal-lysine barley may be closed.

The yield problem must also be tackled at a more molecular level, genetically and biochemically. All identified high-lysine barley variants

are to different extents characterized by an impaired hordein and starch synthesis. This fact suggests that an efficient synthesis of storage products is of significance for a substantial grain filling. As hypothesized by Tsai et al. (1980) maximum yield is acquired only if a maximum amount of storage proteins is synthesized. To understand more about the impact on grain yield of the different storage products in the endosperm, considerably more must be studied regarding the physiology of grain-filling and the possible interrelations between the synthesis of prolamins and starch.

Several investigations have contributed to the knowledge of prolamins synthesis by *in vitro* studies. It is demonstrated that the hordein polypeptides are synthesized on membrane-bound polysomes and vectorially transported into the lumen of endoplasmic reticulum and further transferred into the vacuoles of the cells, where they are deposited in protein bodies (Brandt and Ingversen 1976; Munck and Wettstein 1976; Brandt and Ingversen 1978; Cameron-Mills and Ingversen 1978; Cameron-Mills et al. 1978). The deficiency of hordein proteins in mutant 1508 could be ascribed to abnormalities in the organisation of the membrane-bound polysomes (Brandt and Ingversen 1978). It has been suggested that the mutation is affecting the membrane components involved in protein transport (Ingversen pers. commun.).

Studies of the polymorphism and structural homology of hordein polypeptides are also contributing to an overall understanding of the hordein synthesis and, in a wider sense, of gene control in eucaryotic cells (Doll and Brown 1979; Doll 1980; Jensen et al. 1980; Shewry et al. 1980a; Faulks et al. 1981; Shewry et al. 1981).

Differences in the levels of phytohormones of mutant 1508 compared to Bomi (Mounla et al. 1980) might also provide a new hint for understanding the control of the storage functions of the endosperm, since these phytohormones control multiple physiological and biochemical pathways.

It is concluded that conventional breeding efforts as well as genetic and biochemical studies at the molecular level must be simultaneously conducted in attempts to overcome the low grain yield associated with the high-lysine barley genotypes.

FINAL CONCLUSIONS

Two mechanisms govern the lysine improvement in seeds of high-lysine barley mutants:

1. A decrease of prolamins is compensated for by increased amounts of free lysine and non-prolamin proteins.
2. Specific lysine-rich proteins are increased.

By combining high-lysine genes from each type an additive effect on the lysine content has been achieved.

In these combinations the nutritional quality is improved to meet the essential amino acid requirement by man as well as monogastric animals.

The greatest proportion of seed lysine comes from the high-molecular glutelin proteins to be further studied with regard to function and localization.

Lysine improvement through increased amounts of lysine in the prolamin proteins seems doubtful.

All high-lysine barley genotypes suffer from reduced grain yields.

A normal storage protein synthesis is essential for a normal starch synthesis as some of the high-lysine genes seem to act independently on prolamin synthesis while they probably affect the same pathway in starch synthesis.

To overcome the low grain yields of high-lysine barley it is important that conventional breeding as well as genetic and biochemical studies at a molecular level be simultaneously conducted.

SOME FUTURE PROSPECTS

The emerging use of recombinant DNA methodology may one day provide means for lysine improvement in cereal seeds. A draw-back, so far, has been the inability to form diploid plants from cereal protoplasts in culture. If/when this is attained there will be still another problem,

how to select for a gene in a system where it is not expressed. What is known about the action of the high-lysine genes is confined to the endosperm and it is not expressed in other tissues. However, the enormous experimental advantage to have millions of haploid cells in culture where they can be mutagenized, where selective screens can be applied and where callus tissue and eventually plants can be reconstituted may in the future give positive results for lysine improvement in cereal seeds.

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